STUDIES ON SKIN PERMEABILITY AND HYALURONIDASE INHIBITION IN PREGNANCY AND MENSTRUATION

BY

LENNART PLOMAN

UPSALA 1953

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41548911_0101

STUDIES ON SKIN PERMEABILITY AND HYALURONIDASE INHIBITION IN PREGNANCY AND MENSTRUATION

Akademisk avhandling

som med vederbörligt tillstånd
av vitterfarna Medicinska Fakulteten i Upsala
för vinnande av medicine doktorsgrad
framlägges till offentlig granskning
å Fysiologiska Institutionens föreläsningssal
lördagen den 23 maj 1953 kl. 10 fm.

Av

LENNART PLOMAN
Med. lic., st.

UPPSALA 1953
APPELBERGS BOKTRYCKERI AB

R. UNIV.
BIBLIOTHEEK
LEIDEN

From the Institute of Physiology, University of Upsala, Sweden.

STUDIES ON SKIN PERMEABILITY AND HYALURONIDASE INHIBITION IN PREGNANCY AND MENSTRUATION

BY

LENNART PLOMAN

This communication is a survey of the following papers:

I. *Skin Permeability and Pregnancy.* A Study of Dermal Spread and Absorption in Rabbits and Mice. Acta endocrinol. *10*, 333, 1952.

II. *Human Skin Permeability in Pregnancy and Menstruation.* Absorption Studies by Means of a Photoelectric Dermofluorometer and Dermal Spread Determinations. Acta endocrinol. *13*, Suppl. 14, 1, 1953.

III. *Hyaluronidase Inhibition in Human Pregnancy and Menstruation.* A Study of the Nonspecific Serum Inhibitor. Acta endocrinol. *13*, Suppl. 14, 27, 1953.

These papers will be referred to with their Roman figures, I—III.

INTRODUCTION

The mesenchymal tissues of the female organism are influenced in many respects by conditions such as pregnancy and menstruation. Various changes may be observed — for example, in the dermal connective tissue and in the blood serum.

The extracellular and interfibrillary part of the connective tissue, *i.e., the ground substance,* was described already by *Flemming* in 1876. His work seems to have been overlooked until the findings were recently confirmed by *Day* (1947, 1952). Day has given evidence for the view that the ground substance consists essentially of protein fibrillary networks with the meshes occupied by macromolecules of acid polysaccharides, mainly *hyaluronic acid.* This carbohydrate, which was isolated by *Meyer & Palmer* (1934), plays an important part in the permeability of the connective tissue. It is hydrolyzed and depolymerized with a decrease of viscosity by the enzyme *hyaluronidase (Meyer, Dubos & Smyth,* 1937; *Robertson, Ropes & Bauer,* 1940).

The substrate is also found in the umbilical cord, the synovial fluid and the fluids of the eye *(Meyer,* 1947), and, further, in some bacteria (cf. *Ragan & Meyer,* 1950). The enzyme, which occurs in different forms (cf. *Fishman,* 1951, *Meyer & Rapport,* 1952), is found within the body in the testes and sperma, and, e.g., in various bacteria.

Blood serum has an *inhibitory effect* on the enzymic activity of testis hyaluronidase (*Duran-Reynals,* 1933, *McClean,* 1942). This effect is nonspecific, in contrast to the antibody inhibitors which may appear in the serum following previous contact with a hyaluronidase (*Hobby, Dawson, Meyer & Chaffee,* 1941). The chemical nature of the inhibiting factor (or factors) as well as the mechanism of its action are still obscure. Some evidence indicates that the agent might be a heparin-lipoprotein compound (*Glick & Sylvén,* 1951). The inhibitive process is dependent on several factors, such as temperature, pH, salt concentration, reaction time, and ions present.

As mentioned, the mesenchymal tissues seem to be greatly affected by hormonal factors, though this complex regulatory mechanism is only partly explored as yet. The fact that pregnancy affects the structure and reactions of the connective tissue is evident from the frequent curative temporary effect of pregnancy on rheumatoid arthritis. The influence is similar to the effect achieved with ACTH and cortisone (*Hench,* 1949).

These substances, like epinephrine and various stressor agents (cf. *Selye,* 1950), affect not only the connective tissue. Among their numerous effects on the organism, they have also been found to elevate the serum level of the hyaluronidase inhibitor (cf. *Good, Good, Kelley & Glick*, 1951). The investigators considered their findings indicative of an adrenal cortex mechanism regulating the inhibitor level. On the other hand, the elevated serum levels observed in patients with severe infections (incl. the rheumatic), certain skin diseases, cancer, and other maladies involving the organism as a whole, usually return to normal in response to ACTH and cortisone treatment (*Glick*, 1950, *Good et al*, 1951, *Meyer & Rapport*, 1952).

The present problems. Some of the conditions and findings just outlined, and others not mentioned here, indicated that an investigation of functional changes during pregnancy and menstruation in the dermal connective tissue and the serum hyaluronidase inhibitor would be of interest.

AUTHOR'S RESULTS

Paper I.

The influence of pregnancy on the permeability of the dermal connective tissue was investigated in white *rabbits* and *mice*. Two test methods were used, one of which involved measurements of the spread of a dye injected into the corium, and the other estimations of the disappearance time of a fluorescent dye identically injected. The indicators used were isotonic solutions of *hemoglobin* and *uranin,* respectively, and the intradermal injections were made in the depilated skin of the animals. The areas of the coloured patches of hemoglobin were determined after certain intervals of time. The uranin wheals were observed in a dark room with ultraviolet light and the time was recorded until the fluorescence ceased.

From these experiments it was obvious that *living rabbits in advanced pregnancy exhibited a smaller spread of hemoglobin and a more rapid disappearance of uranin,* both with and

without *hyaluronidase* in the indicator solutions, as compared with non-pregnant rabbits. The spread was also inhibited in killed pregnant rabbits (but not in killed mice), which was taken to indicate a change in the ground substance and not merely in the circulation of the connective tissue.

The inhibited hemoglobin spread was in accordance with the findings of *Ducommun, Timiras & Dordoni* (1951), who observed a similar inhibition in rats towards the end of pregnancy.

When discussing the probable mechanism behind these permeability changes in the connective tissue during pregnancy, three important factors are emphasized: the influence of oestrogenic hormones, the state of the skin fluid, and the stress effects of the gestation.

Paper II

As a development of the animal experiments described above, and to supplement the scanty *human* data available in this field, dermal absorptive and spreading phenomena were studied in normal women during pregnancy and menstruation.

Some investigations of human skin permeability have earlier been carried out in advanced pregnancy (*Lash*, 1926) and during menstruation (*Seeberg*, 1947, 1950). *Lash* used the palpation method of *Aldrich & McClure* (1923, 1924) when studying the absorption from intradermally applied wheals of isotonic NaCl. He found that the transfer from the site of injection of the net volume supplied was considerably accelerated in cases of toxaemia and most rapid in eclampsia. He considered a sudden shortening of the absorption time in a pregnant woman as the earliest sign of an imminent toxaemia. No data for non-pregnant women were given. *Seeberg* found the dermal absorption of various indicators to be more rapid during the premenstrual period and the first few days of menstruation than elsewhere in the cycle.

In order to be independent of the darkened room with a constant luminous intensity for the fluorescence determinations, and to get rid of the subjective factor involed in the method, a *photoelectric dermofluorometer* was constructed. By

means of this apparatus the decay of fluorescence from the wheal could be registered during the absorption.

It consists of a mobile unit containing an ultraviolet light source, an electron multiplier phototube and glass filters, and a fixed unit comprising the DC voltage supply to the phototube and a galvanometer registering the output current from the tube. The apparatus, as well as the treatment of the primary data obtained with it and the corrections for the technical sources of error, are described in detail in the paper.

The fluorescence decay of the uranin wheal was found to follow approximately an exponential time function, and the slope of the logarithmic decay curve was a measure of the rate of the absorptive process.

The intradermal injections were made on the volar side of the forearm, and the fluorescence was measured in 20 normal women in the last two months of pregnancy, 20 on the 1st and 20 on the 8th day of the menstrual cycle. *The uranin disappearance rate in the pregnant women was considerably higher* (the slopes mentioned were steeper) *than in the non-pregnant subjects. At the beginning of menstruation the women exhibited a more rapid decay than one week later in the cycle,* though this difference was not so marked.

Spreading experiments with hemoglobin were carried out on the same subjects. There was practically no difference between the three groups. A plausible reason for this somewhat surprising result may have been the fact that the hemoglobin indicator was not inert for *homo*. Some of the test subjects were sensitive to the injections. This might have concealed differences which would be evident with another indicator.

An investigation with the dermofluorometer of the dermal absorptive behaviour in cases of toxaemic pregnancy seems indicated. That should give an opportunity to test the findings of *Lash*, regarding the absorption rate as a diagnostic and prognostic resource in dealing with toxaemia.

Paper III

The human material reported above, was also tested with regard to the hyaluronidase inhibitive capacity of the blood serum.

Of the methods available for the present study, a modification of the *viscometric* method, introduced by *Madinaveitia & Quibell* (1940), was employed.

Different methods were tried, and the reasons for the choice of method are given in the paper.

A similar investigation of its variations in women has been described by *Hakanson & Glick* (1949). They noticed an insignificant rise of the inhibitor level during menstruation and in the last months of pregnancy. (Only in the puerperium did they observe a marked elevation.)

The reduction in the viscosity of a solution containing hyaluronic acid was measured at constant temperature by means of an Ostwald viscometer. This reduction was accomplished by incubation with hyaluronidase, which in turn had been preincubated with serum. The substrate used was a sodium hyaluronate, prepared from human umbilical cords. The enzyme was obtained from bovine testes and then stabilized with methylated gelatine.

The hyaluronidase inhibition was calculated by means of formulae established by *Staudinger & Heuer* (1930) and *Hultin* (1946). The reciprocal viscosity values within a certain range lay on a straight line when plotted against time. The slope of this line was directly proportional to the drop of viscosity and therefore to the activity of the enzyme. The different slopes (activities) were compared and the hyaluronidase inhibition was expressed as per cent inactivated enzyme.

The experiments showed that *the inhibitory effect of serum on hyaluronidase was higher in the pregnant women than in the non-pregnant*. The inhibition at the beginning of menstruation did not differ significantly from that one week later in the menstrual cycle.

Possible connections between the permeability changes observed in the dermal connective tissue (I, II) and the alterations found in the serum (III) are discussed in the latter paper. Attempts have been made by several investigators to correlate these phenomena, usually with reference to the findings in rheumatic diseases. An important point in this problem is the

alleged occurrence of hyaluronidase in the connective tissue. This question does not seem to have been definitely settled yet. It is therefore impossible to draw any conclusions regarding correlations in these respects from the findings hitherto presented. The biological significance of the anti-hyaluronidase effect of the blood remains obscure.

SUMMARY

I. An inhibited hemoglobin spread and an accelerated uranin absorption were found in the dermal connective tissue of rabbits in late pregnancy. The hormonal regulatory mechanism for the connective tissue is discussed, and the influence of factors such as oestrogens, hydration, and stress are emphasized.

II. Measurements with a photoelectric dermofluorometer showed the fluorescence decay of intradermally injected uranin to be more rapid in late human pregnancy. Fluctuations in these respects were noticed also during the menstrual cycle. An investigation of dermal absorption in toxaemic pregnancies by means of the new apparatus is thought to be of interest.

III. An increased hyaluronidase inhibitory effect of serum in late human pregnancy was observed, using a viscometric method. Possible connections between the changes found in blood and connective tissue are briefly discussed.

ACKNOWLEDGEMENTS

My thanks are due to various members of the scentific staffs at the Physiological Department, as well as the Biochemical, Histological and Pharmacological Departments, for valuable help and criticism.

I am also grateful to Professor John Naeslund for permission to obtain test subjects from the Prenatal Clinic.

REFERENCES

Aldrich, C. A. & McClure, W. B.: J. A. M. A. *82*, 1425, 1924.
Day, T. D.: J. Path. & Bact. *59*, 567, 1947.
Day, T. D.: J. Physiol. *117*, 1, 1952.
Ducommun, P., Timiras, P. S. & Dordoni, F.: Surg., Gyn. & Obst. *93*, 243, *1951*.
Duran-Reynals, F.: J. Exper. Med. *58*, 161, 1933.
Fishman, W. H.: The Enzymes, *1*, 769, 1951. Acad. Press, New York.
Flemming, W.: Arch. mikr. Anat. *12*, 434, 1876.
Glick, D.: Mount Sinai Hosp. J. *17*, 207, 1950.
Glick, D. & Sylvén, B.: Science *113*, 388, 1951.
Good, T. A., Good, R. A, Kelley, V. C. & Glick, D.: Am. J. Physiol. *166*, 555, 1951.
Hench, P. S.: Proc. Staff Meet., Mayo Clin. *24*, 167, 1949.
Hobby, G. L., Dawson, M. H., Meyer, K. & Chaffee, E.: J. Exper. Med. *73*, 109, 1941.
Hultin, E.: Svensk Kem. Tidskr. *58*, 281, 1946.
Lash, A. F.: Surg., Gyn. & Obst. *43*, 40, 1926.
Madinaveitia, J. & Quibell, T. H. H.: Biochem. J. *34*, 625, 1940.
McClean, D.: J. Path. & Bact. *54*, 284, 1942.
McClure, W. B. & Aldrich, C. A.: J. A. M. A. *81*, 293, 1923.
Meyer, K.: Physiol. Rev. *27*, 335, 1947.
Meyer, K., Dubos, R. & Smyth, E. M.: J. Biol. Chem. *118*, 71, 1937.
Meyer, K. & Palmer, J. W.: J. Biol. Chem. *107*, 629, 1934.
Meyer, K. & Rapport, M. M.: Adv. in Enzymol. *13*, 199, 1952.
Ragan, C. & Meyer, K.: Ann. New York Acad. Sc. 52, 1108, 1950.
Robertson, W. B., Ropes, M. W. & Bauer, W.: J. Biol. Chem. *133*, 261, 1940.
Seeberg, G.: Acta dermat.-venereol. *27*, Suppl. 18, 1947.
Seeberg G.: Acta dermat.-venereol. *30*, 231, 1950.
Selye, H.: Stress, Acta, inc., Med. Publ., Montreal, 1950.
Staudinger, H. & Heuer, W.: Ber. Deutsch. Chem. Ges. *63*, 222, 1930.

Uppsala 1953. Appelbergs Boktryckeri AB

UPPSALA 1953
APPELBERGS BOKTRYCKERI AB